

AMENDMENTS TO THE CLAIMS

The listing of claims below replaces all prior versions, and listings, of claims the application.

LISTING OF CLAIMS

Claim 1-30. (canceled)

Claim 31. (amended) ~~A method according to claim 27 in which polynucleotide molecules are sorted in a device for processing a flow of polynucleotide molecules, which device~~ for detecting a particular nucleic acid in a sample, which particular nucleic acid has at least one restriction site, and which method comprises:

- (a) contacting the sample with
 - (i.) a primer that hybridizes to the particular nucleic acid a predetermined distance from the restriction site,
 - (ii.) a polymerase and
 - (iii.) a plurality of nucleotides, so that a complementary nucleic acid is synthesized from the primer at least to the restriction site;
- (b) contacting the complementary nucleic acid with a restriction enzyme under conditions capable of cutting the complementary nucleic acid at the restriction site; and
- (c) detecting a nucleic acid fragment having a particular length equal to the fixed distance by a method that comprises:
 - (i) sorting polynucleotide molecules in the sample according to size in a device for processing a flow of polynucleotide molecules, which device comprises a substrate and an analysis unit, wherein the analysis unit comprises:

- a main channel having a polynucleotide sample inlet, a detection region downstream of the sample inlet and a branch point discrimination region adjacent to and downstream of the detection region, wherein on average one polynucleotide molecule at a time is placed within the detection region;
- at least two branch channels originating at the branch point discrimination region and in communication with the main channel;
- a detector sensitive to polynucleotide molecules passing through the detection region; and
- a flow control responsive to the detector and acting to direct polynucleotide molecules at the discrimination region into a selected branch channel; and

(ii) identifying a polynucleotide having the particular length, wherein the presence of the nucleic acid fragment in the sample indicates that the particular nucleic acid is present in the sample.

Claim 32. (original) A method according to claim 31 wherein the channels of the device are about 1-100 μm in depth.

Claim 33. (original) A method according to claim 31 wherein the detection region of the device has a volume between about 1 femtoliter and 1 nanoliter.

Claim 34. (original) A method according to claim 31 wherein:
at least some of the plurality of nucleotides are detectably labeled, and
polynucleotides are directed to a selected branch channel based on a measured level of the detectable label.

Claim 35. (original) A method according to claim 34 wherein the labeled nucleotides are fluorescently labeled.

Claims 36-40. (canceled)

Claim 41. (amended) A method ~~according to claim 36 in which the nucleic acid fragment is detected~~ for detecting a particular nucleic acid in a sample,
which particular nucleic acid has at least one restriction site, and
which method comprises:

- (a) contacting the sample with a restriction enzyme under conditions
capable of cutting the particular nucleic acid at the restriction site;
- (b) contacting the sample with a primer that hybridizes to the nucleic acid
a predetermined distance from the cut at the restriction site, a
polymerase and a plurality of nucleotides, so that a complementary
nucleic acid fragment is synthesized; and
- (c) detecting the complementary to nucleic acid fragment using a device
for processing a flow of polynucleotide molecules, which device
comprises a substrate and an analysis unit,

wherein the analysis unit comprises:

- a main channel having a polynucleotide sample inlet, a detection region downstream of the sample inlet and an outlet region adjacent to and downstream of the detection region; and
- a detector sensitive to polynucleotides passing through the detector region, and where[[in]], on average, one polynucleotide at a time is placed within the detection region,

wherein the presence of the complementary nucleic acid fragment in the sample indicates that the particular nucleic acid is present in the sample.

Claim 42. (original) A method according to claim 41 wherein the channels of the device are about 1-100 μm in depth.

Claim 43. (original) A method according to claim 41 wherein the detection region of the device has a volume between about 1 femtoliter and 1 nanoliter.

Claim 44. (original) A method according to claim 41 wherein the detector is sensitive to the size of polynucleotide molecules passing through the detection region.

Claim 45. (original) A method according to claim 41 wherein:
at least some of the plurality of nucleotides are detectably labeled; and
the detector is sensitive to the detectable label.

Claim 46. (original) A method according to claim 45 wherein the labeled nucleotides are fluorescently labeled.

Claims 47-50 (canceled)

Claim 51. (original) A method ~~according to claim 47~~ for detecting a particular nucleic acid in a sample,
which particular nucleic acid has at least one restriction site, and
which method comprises:

- (a) contacting the sample with a restriction enzyme under conditions capable of cutting the particular nucleic acid at the restriction site;
- (b) contacting the sample with a primer that hybridizes to the nucleic acid a predetermined distance from the cut at the restriction site, a polymerase and a plurality of nucleotides, so that a complementary nucleic acid fragment is synthesized; and
- (c) detecting the complementary to nucleic acid fragment according to a method that comprises:
 - (i.) sorting polynucleotide molecules in the sample according to size in which polynucleotide molecules are sorted in a device for processing a flow of polynucleotide molecules, which device comprises a substrate and an analysis unit,

wherein the analysis unit comprises:

- a main channel having a polynucleotide sample inlet, a detection region downstream of the sample inlet and a branch point discrimination region adjacent to and downstream of the detection region, wherein on average one polynucleotide molecule at a time is placed within the detection region;
- at least two branch channels originating at the branch point discrimination region and in communication with the main channel;
- a detector sensitive to polynucleotide molecules passing through the detection region; and
- a flow control responsive to the detector and acting to direct polynucleotide molecules at the discrimination region into a selected branch channel.; and

(i.) identifying a polynucleotide having the particular length, wherein the presence of the complementary nucleic acid fragment in the sample indicates that the particular nucleic acid is present in the sample.

Claim 52. (original) A method according to claim 51 wherein the channels of the device are about 1-100 μm in depth.

Claim 53. (original) A method according to claim 51 wherein the detection region of the device has a volume between about 1 femtoliter and 1 nanoliter.

Claim 54. (original) A method according to claim 51 wherein:
at least some of the plurality of nucleotides are detectably labeled, and
polynucleotides are directed to a selected branch channel based on a measured level of the detectable label.

Claim 55. (original) A method according to claim 51 wherein the labeled nucleotides are fluorescently labeled.
